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A THREE-COMPONENT THEORY OF SEDIMENTATION EQUILIBRIUM IN A DENSITY GRADIENT*

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In the original analysis¹ of the behavior of macromolecules and viruses in a density gradient at equilibrium in the ultracentrifuge, only two incompressible components were considered to be present. These were the polymer and the solvent which forms the density gradient. The pressure-dependent terms and the cross terms in the chemical potential which describe the chemical reactions between the polymer and the solute were neglected. It has been pointed out from theoretical considerations²⁻⁴ that these terms are important. In experimental studies of the buoyant behavior of bovine mercaptalbumin in aqueous cesium chloride,⁵ it was observed that both solvation and pressure effects are large.

In the following first-order theory of sedimentation equilibrium in a density gradient, both solvation effects and pressure dependencies are taken into account. It is shown below that a single buoyant macromolecular substance again gives rise to a Gaussian distribution of concentration. From the standard deviation of this distribution the anhydrous and the solvated molecular weight may be obtained provided certain additional ultracentrifuge, partial specific volume, and activity data are collected.

Two succeeding papers deal with the determination of the solvation⁶ and the pressure-dependent parameters⁷ for T-4 bacteriophage deoxyribonucleic acid. The pressure-dependent parameters for tobacco mosaic virus are also discussed.

The thermodynamic equations describing the equilibria in a three-component system in a centrifugal field are⁸

$$M_1(1 - \bar{v}_1\rho)\omega^2 r dr = \left(\frac{\partial\mu_1}{\partial m_1}\right)_{m_3} dm_1 + \left(\frac{\partial\mu_1}{\partial m_3}\right)_{m_1} dm_3; \quad (1)$$

$$M_3(1 - \bar{v}_3\rho)\omega^2 r dr = \left(\frac{\partial\mu_3}{\partial m_3}\right)_{m_1} dm_3 + \left(\frac{\partial\mu_3}{\partial m_1}\right)_{m_3} dm_1. \quad (2)$$

In these equations ρ is the density of the solution and ω is the angular velocity. The subscripts 1 and 3 refer to one of the solutes and to the macromolecule respectively. The symbols M , \bar{v} , μ , and m stand for molecular weight, partial specific volume, chemical potential, and molality. These differential equations are valid at constant temperature and pressure. The solvation parameter⁹⁻¹¹ $\Gamma \equiv -(\partial\mu_1/\partial m_3)_{m_1}/(\partial\mu_1/\partial m_1)_{m_3}$ represents the net solvation of the polymer in moles solute per mole polymer and is equal to $(\partial m_1/\partial m_3)_\mu$ by the triple product rule.

The quantity Γ is the number of moles of solute 1 which must accompany the addition of one mole of macromolecule to a very large volume of solution if this addition is to be at constant chemical potential, μ . Equations (1) and (2) are transformed to the more useful equations (3) and (4) upon substitution of the defined quantity Γ and the further relation³ $(\partial\mu_1/\partial m_3)_{m_1} = (\partial\mu_3/\partial m_1)_{m_3}$. The relation between the cross terms is valid if concentrations are expressed in molalities.

$$M_1(1 - \bar{v}_1\rho)\omega^2 r dr = \left(\frac{\partial\mu_1}{\partial m_1}\right)_{m_3} dm_1 - \Gamma \left(\frac{\partial\mu_1}{\partial m_1}\right)_{m_3} dm_3; \quad (3)$$

$$M_3(1 - \bar{v}_3\rho)\omega^2 r dr = \left(\frac{\partial\mu_3}{\partial m_3}\right)_{m_1} dm_3 - \Gamma \left(\frac{\partial\mu_1}{\partial m_1}\right)_{m_3} dm_1. \quad (4)$$

Focusing attention first on the polymer, we eliminate dm_1 from equations (3) and (4).

$$[(M_3 + \Gamma M_1) - (M_3\bar{v}_3 + \Gamma M_1\bar{v}_1)\rho]\omega^2 r dr = \left[\left(\frac{\partial\mu_3}{\partial m_3}\right)_{m_1} - \Gamma^2 \left(\frac{\partial\mu_1}{\partial m_1}\right)_{m_3}\right] dm_3. \quad (5)$$

The solvation parameter may be defined on a weight basis, $\Gamma' = \Gamma(M_1/M_3)$. Rearranging equation (5) leads to

$$M_3(1 + \Gamma') \left[1 - \left(\frac{\bar{v}_3 + \Gamma'\bar{v}_1}{1 + \Gamma'}\right)\rho\right] \omega^2 r dr = \left(\frac{\partial\mu_3}{\partial m_3}\right)_{m_1} \left[1 - \frac{(\partial\mu_1/\partial m_3)_{m_1}^2}{(\partial\mu_1/\partial m_1)_{m_3}(\partial\mu_3/\partial m_3)_{m_1}}\right] dm_3. \quad (6)$$

At the position of the maximum polymer concentration, $(dm_3/dr) = 0$. With this position defining band center, denoted here with the subscript zero, the buoyancy condition from equation (6) is³

$$\frac{1}{\rho_0} = \frac{\bar{v}_3 + \Gamma'\bar{v}_1}{1 + \Gamma'}. \quad (7)$$

Clearly the experimentally determined buoyant density ρ_0 is that of the solvated polymer. It should be emphasized that up to this point no assumptions have been made. The density gradient procedure provides a method for determining Γ' if \bar{v}_1 and \bar{v}_3 are known.

If we are to obtain the polymer distribution, the right side of equation (10) must be evaluated. The term, $(\partial\mu_1/\partial m_3)_{m_1}^2/(\partial\mu_1/\partial m_1)_{m_3}(\partial\mu_3/\partial m_3)_{m_1}$, which will be referred to as ϵ , is interesting. It is a general condition of stability with respect to the formation of a new phase that ϵ be less than one. To estimate an order of magnitude of ϵ , all the terms are assumed to be ideal.

$$\epsilon_i = \Gamma'^2 \left(\frac{M_3}{M_1}\right)^2 \frac{RT/m_1}{RT/m_3} = \Gamma'^2 \left(\frac{M_3}{M_1}\right) \left(\frac{w_3}{w_1}\right), \quad (8)$$

where $w = Mm$.

At common experimental conditions for DNA and proteins in CsCl, ϵ_i takes on values between 0.2 and 8 at band center without the formation of a new phase.

Because this term is concentration-dependent, the foregoing ideality assumption predicts non-Gaussian concentration distributions.

In density gradient experiments this ideality assumption is certainly not valid, despite the very low DNA concentrations usually used. Instead, independent solvated molecules are suggested by the observed Gaussian distributions. At low polymer concentrations, the solvated molecules behave independently only at constant solute chemical potential. The assumption of independent solvated particles may be stated:

$$\left(\frac{\partial\mu_3}{\partial m_3}\right)_{\mu_1} = \left(\frac{\partial\mu_3}{\partial m_3}\right)_{m_1} + \left(\frac{\partial\mu_3}{\partial m_1}\right)_{m_3} \left(\frac{\partial m_1}{\partial m_3}\right)_{\mu_1} = \frac{RT}{m_3}. \quad (9)$$

Substituting the relations for Γ into this equation, we obtain

$$\left(\frac{\partial\mu_3}{\partial m_3}\right)_{\mu_1} = \left(\frac{\partial\mu_3}{\partial m_3}\right)_{m_1} - \Gamma^2 \left(\frac{\partial\mu_1}{\partial m_1}\right)_{m_3} = \frac{RT}{m_3}. \quad (10)$$

Combining equations (5) and (6) with (10) gives an equation for the independent solvated macromolecule:

$$M_s[1 - \bar{v}_s\rho]\omega^2 r dr = \frac{RT}{m_3} dm_3 \quad (11)$$

where $M_s = M_3(1 + \Gamma')$ and $\bar{v}_s = \frac{\bar{v}_3 + \Gamma'\bar{v}_1}{1 + \Gamma'}$.

The polymer introduces another interesting problem. Equation (11) has two solutions. If the solute, 1, is water, Γ' is positive; if the solute is salt, Γ' is negative. One solution yields a value of M_3 less than M_s ; the other, greater. This is physically reasonable because the system cannot distinguish between dry polymer, and polymer plus an amount of solution of density $1/\bar{v}_3$ which contains Γ moles of salt per mole of polymer. To obtain the anhydrous molecular weight, Γ' must be positive. In the experimental work on DNA⁶ all molalities are expressed as moles per 1000 gm of salt. In this case Γ is positive and represents moles water bound per mole polymer. M_3 becomes the molecular weight of the dry polymer.

Reexamining the value of ϵ under the assumption of independent solvated particles expressed above, we find that

$$\epsilon = \frac{\Gamma^2(\partial\mu_1/\partial m_1)_{m_3}}{(\partial\mu_3/\partial m_3)_{m_1}} = \frac{\Gamma^2(\partial\mu_1/\partial m_1)_{m_3}}{RT/m_3 + \Gamma^2(\partial\mu_1/\partial m_1)_{m_3}},$$

and ϵ will now always be less than one.

The final question to be answered by the theory is the effect of solvation on the density gradient. Upon elimination of dm_3 from equations (3) and (4), equation (12) is obtained:

$$\frac{dm_1}{dr} = \frac{[(M_1 + \gamma\Gamma M_3) - (M_1\bar{v}_1 + \gamma\Gamma M_3\bar{v}_3)\rho]\omega^2 r}{(\partial\mu_1/\partial m_1)_{m_3}[1 - \Gamma^2\gamma]} \quad (12)$$

where $\gamma = (\partial\mu_1/\partial m_1)_{m_3}/(\partial\mu_3/\partial m_3)_{m_1}$. Although hydrated water represents a very small fraction of the total water, it makes a large contribution to the water-

concentration gradient. Free water alone, however, determines the density gradient because the hydrated polymer has a density almost identical with that of the solution. The effect of the hydrated polymer on the density gradient is therefore negligible. The gradient of free water can be expressed by the equation $(dm_1/dr)_t = (dm_1/dr) - \Gamma(dm_3/dr)$. Substituting equations (5) and (12) into this expression, we obtain

$$\left(\frac{dm_1}{dr}\right)_t = \frac{M_1(1 - \bar{v}_1\rho)\omega^2r}{(\partial\mu_1/\partial m_1)_m}. \quad (13)$$

Effects of polymer on the activity of the solvent are completely negligible at the polymer concentrations normally used. Equation (13) is the condition for equilibrium in a two-component system. It therefore does not matter that the polymer is present nor which definition of molality is used in evaluating the density gradient.

In order to obtain an expression for the polymer distribution at equilibrium, ρ , \bar{v}_s , and M_s are expanded about band center. $\delta = r - r_0$.

$$\rho = \rho_0 + \left(\frac{d\rho}{dr}\right)\delta, \quad \bar{v}_s = \bar{v}_{s,0} + \left(\frac{d\bar{v}_s}{dr}\right)\delta, \quad M_s = M_{s,0} + \left(\frac{dM_s}{dr}\right)\delta$$

Substituting these equations into equation (11), using the buoyancy condition $(1 - \bar{v}_{s,0}\rho_0) = 0$ and keeping only first-order terms in δ , we obtain

$$-M_{s,0} \left[\bar{v}_{s,0} \left(\frac{d\rho}{dr}\right) + \rho_0 \left(\frac{d\bar{v}_s}{dr}\right) \right] \delta \omega^2 r_0 d\delta = RT d \ln m_3. \quad (14)$$

Integrating equation (14), we obtain a distribution of the form $m = m_0 \exp(-\delta^2/2\sigma^2)$ where σ^2 is given by equations (15) and (16).¹²

$$\sigma^2 = \frac{RT}{M_{s,0} \bar{v}_{s,0} (d\rho/dr)_{\text{eff}} \omega^2 r_0} \quad (15)$$

$$\left(\frac{d\rho}{dr}\right)_{\text{eff}} = \left(\frac{d\rho}{dr}\right) + \frac{\rho_0}{\bar{v}_{s,0}} \left(\frac{d\bar{v}_s}{dr}\right) \quad (16)$$

From the experimental evidence presented in references 6 and 7, \bar{v}_s is known to be a function of pressure and solute activity at atmospheric pressure, a_1^0 . The following equations allow the separation of these two effects and define an apparent compressibility for the solvated polymer, κ_s .

$$\frac{d\bar{v}_s}{dr} = \left(\frac{\partial\bar{v}_s}{\partial P}\right)_{a_1^0} \left(\frac{dP}{dr}\right) + \left(\frac{\partial\bar{v}_s}{\partial a_1^0}\right)_P \left(\frac{da_1^0}{dr}\right). \quad (17)$$

$$\kappa_s = -\frac{1}{\bar{v}_s} \left(\frac{\partial\bar{v}_s}{\partial P}\right)_{a_1^0}. \quad (18)$$

To express the second term on the right-hand side of equation (17) in terms of buoyant densities, the expression $1/\bar{v}_s = \rho_s$ which defines ρ_s is differentiated with respect to a_1^0 . Neglecting higher-order terms, this results in

$$-\frac{1}{\bar{v}_{s,0}} \left(\frac{d\bar{v}_s}{da_1^0}\right) = \frac{1}{\rho_{s,0}} \left(\frac{d\rho_s}{da_1^0}\right). \quad (19)$$

Now $(d\rho_s/da_1^0) = (\partial\rho_0^0/\partial a_1^0)_P$. This quantity is a slope which can be experimentally determined from the buoyant density versus water activity in several aqueous cesium salt solutions.⁶ This operation assumes that the anion has no effect on the buoyant density except by way of its effect on the water activity.

Upon substituting equations (17), (18), and (19) into equation (16), the effective density gradient becomes

$$\left(\frac{d\rho}{dr}\right)_{\text{eff}} = \left(\frac{d\rho}{dr}\right) - \rho_0\kappa_s \frac{dP}{dr} - \left(\frac{\partial\rho_0^0}{\partial a_1^0}\right)_P \left(\frac{da_1^0}{dr}\right). \quad (20)$$

In order to obtain a workable buoyancy condition, it is necessary to expand \bar{v}_s about atmospheric pressure, P equal to zero. The activity of the solute a_1^0 , and the density of the solution ρ^0 at atmospheric pressure are not independent. It is therefore possible to expand in terms of ρ^0 instead of a_1^0 . The expansion is given by equation (21).

$$\bar{v}_{s,0} = \bar{v}_{s,0}^0 \left[1 - \kappa_s P_0 - \frac{1}{\rho_0^0} \left(\frac{\partial\rho_0^0}{\partial a_1^0} \right)_P \left(\frac{da_1^0}{d\rho^0} \right) \left(\rho_0^0 - \frac{1}{\bar{v}_{s,0}^0} \right) \right]. \quad (21)$$

Using the relation $\rho_0 = \rho_0^0/(1 - \kappa P_0)$, the buoyancy condition, $\bar{v}_{s,0} \rho_0 = 1$, can now be written as follows:

$$\rho_0^0 = \frac{1}{\bar{v}_{s,0}^0} \left[1 - \frac{(\kappa - \kappa_s)P_0}{1 - \left(\frac{\partial\rho_0^0}{\partial a_1^0} \right)_P \left(\frac{da_1^0}{d\rho^0} \right)} \right], \quad (22)$$

where $\bar{v}_{s,0}^0$ is the reciprocal of the buoyant density at atmospheric pressure. Higher-order corrections throughout this discussion have been neglected. It is shown in reference 7 that the physical density gradient is given by equation (23):

$$\frac{d\rho}{dr} = \left[\frac{1}{\beta^0} + \kappa\rho^{02} \right] \omega^2 r. \quad (23)$$

The complete effective density gradient is obtained by substituting equation (23) into equation (20). Higher-order corrections are again neglected and the a_1^0 dependence is expressed in terms of the density at atmospheric pressure as a concentration variable. The substitution $dP/dr = \rho\omega^2 r$ (10) has also been made.

$$\left(\frac{d\rho}{dr}\right)_{\text{eff}} = \left[\frac{1}{\beta^0} + \frac{(\kappa - \kappa_s)\rho^{02}}{1 - \left(\frac{\partial\rho_0^0}{\partial a_1^0} \right)_P \left(\frac{da_1^0}{d\rho^0} \right)} \right] \left[1 - \left(\frac{\partial\rho_0^0}{\partial a_1^0} \right)_P \left(\frac{da_1^0}{d\rho^0} \right) \right] \omega^2 r. \quad (24)$$

All the terms of this equation can be experimentally determined. The quantity

$$(\kappa - \kappa_s)/[1 - (\partial\rho_0^0/\partial a_1^0)_P (da_1^0/d\rho^0)] = \Psi$$

can be determined by studying the pressure dependence of the buoyant density, as can be seen from equation (22). The quantity $(\partial\rho_0^0/\partial a_1^0)_P$ is the slope of buoyant density versus solute activity at P equals zero. The quantity $(da_1^0/d\rho^0)$ is the slope of solute activity versus density for the salt solution at P equal zero.

Upon multiplying as indicated in equation (24), and recalling that the composition density gradient $d\rho^0/dr = \omega^2 r/\beta^0$, we find that the effective density gradient is

$$\left(\frac{d\rho}{dr}\right)_{\text{eff}} = \left(\frac{d\rho^0}{dr}\right) + (\kappa - \kappa_s)\rho_0^2\omega^2r - \left(\frac{\partial\rho_0^0}{\partial a_1^0}\right)_P \left(\frac{da_1^0}{d\rho^0}\right) \left(\frac{d\rho^0}{dr}\right) \quad (25)$$

or

$$\left(\frac{d\rho}{dr}\right)_{\text{eff}} = \left(\frac{d\rho^0}{dr}\right) - \left(\frac{\partial\rho_s^0}{\partial r}\right)_P + (\kappa - \kappa_s)\rho_0^2\omega^2r.$$

For convenience, $(\partial\rho_s^0/\partial r)_P$ is set equal to $(\partial\rho_0^0/\partial r)_P$, defined as above.

The effective density gradient is now seen to be the composition density gradient diminished by the polymer density gradient associated with solvation changes and enhanced by an effective compression density gradient. This latter density gradient is the result of the difference in compressibilities of solvent and the solvated polymer. The effective density is thus in the appropriate form for the physical situation.

The solvated macromolecules are everywhere in the band in equilibrium with the layer of solution perpendicular to the field. In each layer, the hydrated macromolecules are differently solvated and in addition differently compressed.

Appendix.—It can be shown that the assumption that $(\partial\mu_3/\partial m_3)_{\mu_1} = RT/m_3$ is consistent with the idea that Γ is independent of polymer concentration at constant μ_1 . Partial differentiation at constant μ_1 of the expression defining Γ , $(\partial\mu_3/\partial m_1)_{m_3} = -\Gamma(\partial\mu_1/\partial m_1)_{m_3}$ with respect to m_3 gives

$$\begin{aligned} -\frac{\partial}{\partial m_3} \left[\left(\frac{\partial\mu_3}{\partial m_1} \right)_{m_3} \right]_{\mu_1} &= \left(\frac{\partial\Gamma}{\partial m_3} \right)_{\mu_1} \left(\frac{\partial\mu_1}{\partial m_1} \right)_{m_3} + \Gamma \frac{\partial}{\partial m_3} \left[\left(\frac{\partial\mu_1}{\partial m_1} \right)_{m_3} \right]_{\mu_1}; \\ \frac{\partial}{\partial m_3} \left[\left(\frac{\partial\mu_3}{\partial m_1} \right)_{m_3} \right]_{\mu_1} &= \frac{\partial}{\partial m_1} \left[\left(\frac{\partial\mu_3}{\partial m_3} \right)_{\mu_1} \right]_{m_3} = \frac{\partial}{\partial m_1} \left(\frac{RT}{m_3} \right)_{m_3} = 0; \\ \frac{\partial}{\partial m_3} \left[\left(\frac{\partial\mu_1}{\partial m_1} \right)_{m_3} \right]_{\mu_1} &= \frac{\partial}{\partial m_1} \left[\left(\frac{\partial\mu_1}{\partial m_3} \right)_{\mu_1} \right]_{m_3} = 0. \end{aligned}$$

Therefore $(\partial\Gamma/\partial m_3)_{\mu_1}(\partial\mu_1/\partial m_1)_{m_3} = 0$. For stability $(\partial\mu_1/\partial m_1)_{m_3} > 0$. Therefore, $(\partial\Gamma/\partial m_3)_{\mu_1} = 0$.

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